Appetite 96 (2016) 254–259

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Exogenous glucagon-like peptide-1 acts in sites supplied by the cranial mesenteric artery to reduce meal size and prolong the intermeal interval in rats

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ARTICLE INFO

Article history:
Received 10 June 2015
Received in revised form
24 September 2015
Accepted 25 September 2015
Available online 30 September 2015

Keywords:
GLP-1
Intermeal interval
Satiety ratio
Celiac artery
Portal vein

ABSTRACT

Three experiments were done to better assess the gastrointestinal (GI) site(s) of action of GLP-1 on food intake in rats. First, near-spontaneous nocturnal chow meal size (MS), intermeal intervals (IMI) length and satiety ratios (SR = MS/IMI) were measured after infusion of saline, 0.025 or 0.5 nmol/kg GLP-1 into the celiac artery (CA, supplying the stomach and upper duodenum), cranial mesenteric artery (CMA, supplying small and all of the large intestine except the rectum), femoral artery (FA, control) or portal vein (PV, control). Second, infusion of 0.5 nmol/kg GLP-1 was tested after pretreatment with the GLP-1 receptor (GLP-1R) antagonist exendin-4(3–39) via the same routes. Third, the regional distribution of GLP-1R in the rat GI tract was determined using rtPCR. CA, CMA and FA GLP-1 reduced first MS relative to saline, with the CMA route more effective than the others. Only CMA GLP-1 prolonged the IMI. None of the infusions affected second MS or later eating. CA and CMA GLP-1 increased the SR, with the CMA route more effective than the CA route. CMA exendin-4 (3–39) infusion reduced the effect of CMA GLP-1. Finally GLP-1R expression was found throughout the GI tract. The results suggest that exogenous GLP-1 acts in multiple GI sites to reduce feeding under our conditions and that GLP-1R in the area supplied by the CMA, i.e., the small and part of the large intestine, plays the leading role.

1. Introduction

Glucagon-like Peptide-1 (7–36)-amide (GLP-1) is a 30-amino acid peptide secreted by the L-cells of the gastrointestinal (GI) tract as well as some pancreatic cells and a small population of neurons in the nucleus tractus solitaries. Intestinal GLP-1 is secreted in response to neuroendocrine stimulation and macronutrient sensing. Through activation of a G-protein coupled receptor, GLP-1 receptor (GLP-1R), GLP-1 evokes responses including stimulation of insulin secretion, inhibition of gastric emptying and reduction of food intake (reviewed in (Holst, 2007)).

Evidence suggests that GLP-1 acts both peripherally and centrally to reduce food intake. In support of a peripheral action, systemic administration of albugon, a GLP-1-albumin fusion protein that does not cross the blood–brain barrier, reduced food intake (Baggio, Huang, Brown, & Drucker, 2004) and intraperitoneal injections of the GLP-1R antagonist exendin (9–39) increased food intake (Asarian et al., 2012; Williams, Baskin, & Schwartz, 2009). In addition, total abdominal vagotomy or selective subdiaphragmatic vagal deafferentation attenuated the reduction of food intake by peripherally administered GLP-1 under some conditions (Abbott et al., 2005; Ruttimann, Arnold, Hillebrand, Geary, & Langhans, 2009). On the other hand, in support of a central action, central injection of GLP-1 also decreased food intake, and central injection of exendin (9–39) increased it (Kinzig, D'Alessio, & Seeley, 2002; McMahon & Wellman, 1998; Turton et al., 1996).

The peripheral sites where GLP-1 acts to reduce food intake are unknown. Rüttiman et al. found that GLP-1 infused via intraperitoneal catheters reduced the size of dark-onset meals in rats, whereas GLP-1 infused via hepatic portal-vein catheters did not, indicating that GLP-1R in the GI tract, pancreas or other extrahepatic gut site mediated the effect. Therefore, to further specify the site(s) of action of peripheral GLP-1 on food intake, we compared the effects of GLP-1 infusions into (1) the celiac artery (CA), which supplies the stomach, upper duodenum, part of the

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http://dx.doi.org/10.1016/j.appet.2015.09.030
0195-6663/

pancreas and liver, (2) the cranial mesenteric artery (CMA), which supplies the duodenum, jejunum, ileum, cecum and colon (Robert, 1971; Roger, Cabanie, & Ferre, 1991; Sayegh, 2013a, 2013b; Snipes, 1981), (3) the femoral artery (FA), and (4) the hepatic portal vein (PV). Infusions were done just prior to the onset of the dark cycle, and near-spontaneous meal patterns were measured for 24 h. This represents the first test of the differential feeding effects of GLP-1 infused via different vascular routes. In addition to the better anatomical specificity, this technique permits the use of smaller doses of GLP-1 than necessary in intraperitoneal-injection tests, which may minimizes possible side effects. We previously used this intra-arterial catheterization technique to test CA and CMA infusions of cholecystokinin (CCK) and gastrin releasing peptide (GRP) (Sayegh et al., 2015; Washington, Aglan, & Sayegh, 2014).

In a second experiment, we tested the effects of pretreated with the GLP-1 receptor antagonist exendin-4 (3–39) on the feeding effect of GLP-1 infusions into the CA, CMA, FA and PV. Finally, to support our hypothesis that GLP-1 acts in specific GI loci to control feeding, we determined GLP-1R expression in eleven different GI regions. Dunphy et al. (Dunphy, Taylor, & Fuller, 1998) previously identified GLP-1R in the rat GI tract, but did not provide information about differential expression in different GI loci.

2. Materials and methods

The Tuskegee University Animal Care and Use Committee approved all animal protocols. Adult male Sprague Dawley rats weighing 400–450 g (n = 28, divided into CA, CMA, FA and PV groups, n = 7 each) were individually housed in the BioDAQ E2 system (Research Diets, New Brunswick, NJ) in a controlled environment (12 h dark/12 h light cycle – lights off at 1800 h, 21.5° C), with water and pelleted rodent chow (Teklad, Madison, WI) available *ad libitum*.

2.1. Vascular catheterization

One catheter was implanted in each rat, as described previously (Sayegh et al., 2015; Washington et al., 2014). Catheters (Micro-Renathane R-ITC-SP 9.5, Braintree Scientific, Braintree MA) were 24 cm long. The intravascular portion of the catheter was 0.25 mm OD x 0.12 mm ID, and the size of the remaining part was 0.84 mm OD x 0.36 mm ID. Catheterizations were performed using a surgical microscope (Carl Zeiss Opmi 160 12.5x/18B, 1x250, *Monument*, *CO*). General anesthesia, indicated by the absence of a pedal withdrawal reflex, was achieved with intramuscular injection of 1 ml/kg body weight of a mixture of 5.0 ml of Ketaset [100 mg/kg], 2.5 ml of Rompun® [xylazine 20 mg/kg], *Bayer*, *Shawnee Mission*, *KS*, 1.0 ml of acepromazine maleate® [10 mg/kg], *Bayer*, *Shawnee Mission*, *KS* and 1.5 ml of saline. The abdominal wall was clipped and cleaned with three alternating betadine solution and alcohol swabs. A ventral midline celiotomy was performed.

The CA was exposed and a temporary ligation was placed near the branch point from the aorta to prevent bleeding. The CA was punctured with a sterile 30 gauge needle 1–2 mm distal to this ligature, and the catheter was threaded into the artery and fixed in place using cyanoacrylate glue. The temporary ligation was removed, and the catheter was threaded out of the abdominal cavity subcutaneously, exteriorized between the scapulae and secured with sutures and cyanoacrylate glue. The CMA was similarly catheterized. The FA was exposed on the medial aspect of the right thigh, freed from the surrounding fat and connective tissue, clamped (MC6 double clamp 0.9 cm, Microsurgery Instruments, Inc. Bellaire, TX), and catheterized similarly. The PV was located and exposed on the ventral aspect of the liver and similarly catheterized.

The muscles of the abdominal wall were closed using a polydioxanone II (4-0) absorbable suture in a simple continuous pattern, and the skin was closed using surgical staples. Post-operative care included Metacam® (Meloxicam® [1.1 mg/kg]) subcutaneously for pain control, *Boehringer Ingelheim*, *St. Joseph*, *MO* and Baytril® (Enrofloxacin® [0.05 ml], *Bayer*, *Shawnee Mission*, *KS*) intramuscularly as an anti-bacterial medication, each given daily for 5 d. Rats were allowed two weeks of recovery time. The criteria for complete recovery following surgery included the absence of clinical signs (e.g., signs of pain, porphyria secretion, cold extremities, lethargy) and the return of food intake to pre-operative levels. Catheters were flushed twice daily (0900 h and 1700 h) with 0.3 ml heparinized saline.

The patency of CA and CMA catheters was confirmed during surgery, first, by injecting 0.5 ml sterile saline into the catheters and verifying pallor in the perfused tissue, and, second, by injecting 0.5 ml methylene blue and verifying dye in the perfused tissues. In addition, at the end of the experiment, all rats were sacrificed with an overdose of pentobarbital, and the catheters were infused with latex, whose distribution was verified. Verification of the PV and FA were done by injecting latex only.

2.2. Meal patterns

The BioDAQ E2 Food and Water Intake system detects brief episodes of food intake while minimizing food spillage and hoarding and generates a computerized data stream including times of the initiation of intake activity, the period of the activity, and the weight consumed. The criterion for a meal was consumption of ≥0.2 g, and the criterion for intermeal interval (IMI) was no feeding activity for ≥ 15 min.

After two weeks of recovery from surgery, rats were habituated to the laboratory environment and the experimental design daily for two weeks. For the dose-response experiment, at 1700 h, 1 h before lights off, feeder gates were closed each rat was weighed, handled for a few minutes and received a 0.3 ml infusion of heparinized saline into its catheter. At 1800 h, lights were off, feeder gates were opened. For the antagonist experiment, the rats received two 0.3 ml infusions of heparinized saline, at 1750 h and at 1800 h. First MS, IMI and SR were determined and formed individual baselines for each of rats. These were compared later with the experimental data. If these did not match within two standard deviations, they were not included in the statistical analysis. Four of 28 rats were excluded from the dose-response experiment on this basis. All rats were included in the analysis of the antagonist study.

On Mondays, Wednesdays and Thursdays at 1800 h for the dose-response experiment and at 1750 h for the antagonist experiment, rats received a heparinized saline infusion. On Tuesdays, Thursdays and Saturdays, the dose-response rats received infusions of GLP-1(7-36) (GLP-1; 0, 0.025, 0.5 nmol/kg; Bachem, Torrance, CA, USA) at 1800 h and the antagonist rats received exendin-4 (3–39) (0 or 0.1 mg/kg; Bachem) at 1750 h followed by GLP-1 (0.5 nmol/kg) at 1800 h. Sundays were reserved for the maintenance, but the catheters were flushed with 0.3 ml of the heparinized saline solution twice a day including Saturdays and Mondays. Treatments were done in random order.

2.3. GLP-1R rtPCR

rtPCR was done as previously (Gulley et al., 2005; Lateef et al., 2012). Ten free-fed rats were sacrificed with an overdose of sodium pentobarbital (100 mg/kg, i.p.). Approximately 200 mg (1 cm^2) samples were collected from the esophagus (mid-cervical region), gastric antrum and cardia, pylorus, proximal duodenum (0.5 cm aborad from the pylorus), distal duodenum (5 cm aborad